IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

		Atty. Docket: KARAOLIS1A
In re Application of:	)	Conf. No.: 2282
David K. R. KARAOLIS	)	Art Unit: 1645
Appln. No.: 10/565,591	)	Examiner: N. Archie
Filed: October 6, 2006	)	Washington, D.C.
For: METHOD FOR ATTENTUATING…	)	

DECLARATION UNDER 37 CFR §1.132

Honorable Commissioner for Patents
U.S. Patent and Trademark Office
Customer Service Window
Randolph Building, Mail Stop
401 Dulany Street
Alexandria, VA 22314

Sir:

I David K. R. KARAOLIS, hereby declare and state as follows:

I am the same David K. R. Karaolis listed in the above-identified application as the sole inventor and my educational and professional experience is presented in the curriculum vitae attached hereto.

The experiments presented below and in the attachments to this declaration demonstrate that cyclic di-GMP, or a cyclic dinucleotide, can attenuate the virulence of microbial pathogens, inhibit or reduce microbial colonization and treat an infection. These experiments were conducted

either by me or under my supervision or direction and I can attest of my own personal knowledge that all the results reported herein and in the attachments to this declaration are true and accurate.

Attached hereto as Exhibits 1-3 are copies of Karaolis et al., "Bacterial c-di-GMP Is an Immunostimulatory Molecule", *J. Immunol.* 178:2171-2181, 2007 (Exhibit 1), Karaolis et al., "Cyclic Di-GMP Stimulates Protective Innate Immunity in Bacterial Pneumonia", *Infection and Immunity* 75(10):4942-4950, 2007 (Exhibit 2), Ogunniyi et al., manuscript entitled "c-di-GMP Is an Effective Immunomodulator and Immunostimulatory Molecule Against Pneumococcal Infection" (Exhibit 3). The following highlights of the experimental results from Exhibits 1-3 as well as other unpublished data not shown at this time are summarized below, with citation to Exhibits 1-3 and to data not shown. Please note that the numbering used for the pages of the Exhibits referred to in the following highlights counts from the first page of the Exhibit rather than the actual page number found at the top or bottom of each page.

***Effect of cyclic dinucleotides on microbial virulence and colonization*:**

- Intramammary treatment of mice with c-di-GMP at 12 h and 6 h before challenge with *Staphylococcus aureus* bacteria

gave a protective effect, inhibits colonization and inhibits infection with a 10,000-fold reduction in CFUs in tissues ($p < 0.001$). See Exhibit 1, Abstract, lines 3-4 and page 4, column 2, lines 23-28.

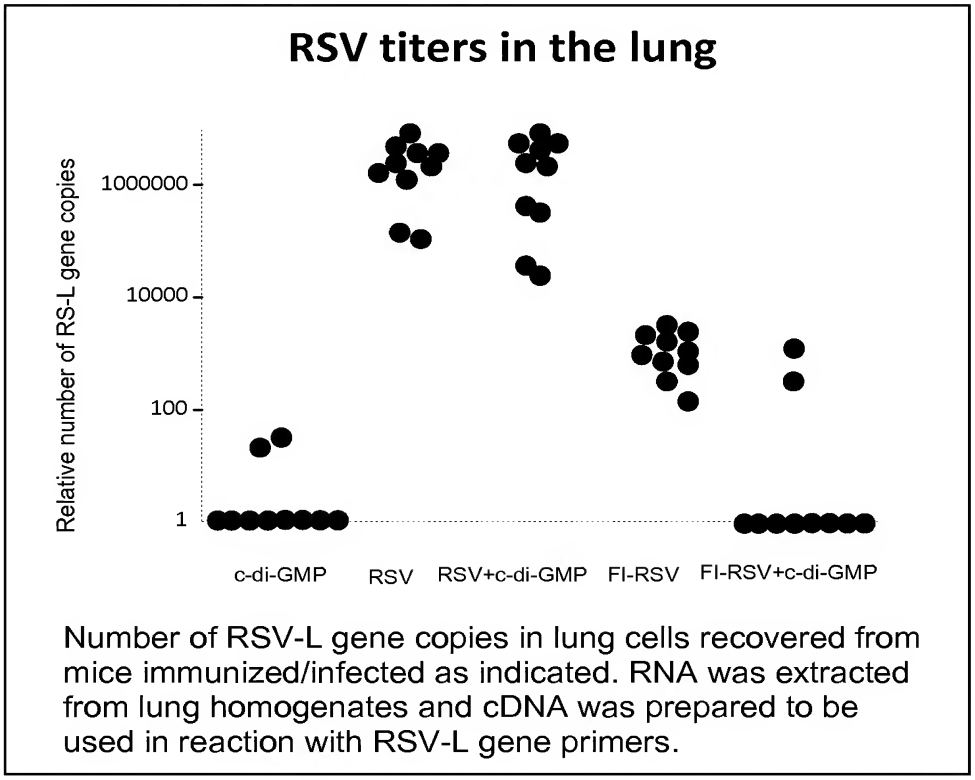
- Local intranasal (i.n.) or systemic subcutaneous (s.c.) administration of c-di-GMP alone prior to intratracheal (i.t.) challenge with *Klebsiella pneumoniae* bacteria stimulates a protective effect, inhibits colonization and inhibits infection and results in significantly increased survival. See Exhibit 2, Abstract, lines 4-6 and page 3, column 2, lines 1-5.
- Pretreatment with c-di-GMP alone results in a 5-fold reduction in *Klebsiella pneumoniae* bacteria in the lung ($P < 0.05$) and an impressive >1,000-fold decrease in bacteria and infection in the blood ($P < 0.01$). See Exhibit 2, Abstract, lines 8-10 and page 4, column 1, lines 5-13.
- Intranasal pretreatment with c-di-GMP alone 48 h and 24 h prior to challenge with *Streptococcus pneumoniae* (pneumococcus) bacteria resulted in significant decrease in bacterial load and infection in the lungs and blood after serotypes 2 and 3 challenge, and significant decrease in lung titers and infection after serotype 4 challenge. See Exhibit 3, Summary, lines 9-11 and page 10, lines 1-5.
- c-di-GMP delivered in either a compartmentalized or systemic fashion stimulates a protective effect, inhibits colonization and inhibits infection in the lung and protects against bacterial invasion. See Exhibit 2, Abstract, lines 14-16.
- c-di-GMP pretreatment by the intranasal (i.n.) route 1 day before and 4 days after *Pneumocystis carinii* (fungal

parasite) infection results in significant clearance in mice (data not shown).

- In conclusion, c-di-GMP can significantly inhibit microbial colonization, virulence and infection against intranasal (i.n.) or intraperitoneal (i.p.) challenge against various bacteria and fungal parasites in different animal models of infection. These experimental results provide support for the administration of cyclic dinucleotides, according to the presently claimed invention, that attenuates the virulence of microbial pathogens, inhibits or reduces microbal colonization and inhibits infection against a variety of bacteria and fungal parasites including *S. aureus*, *K. pneumoniae*, *S. pneumoniae* and *P. carinii*.

Effect of cyclic dinucleotides on inhibting Respiratory Syncytial Virus (RSV) virulence and infection

Mice were pretreated by intramuscular (i.m.) injection with either c-di-GMP alone, RSV alone, Formalin inactivated RSV (FI-RSV) vaccine (50 μl standard dose), RSV + c-di-GMP, or FI-RSV + c-di-GMP two weeks prior to challenge with RSV (10^6 pfu standard challenge dose) given by the standard intranasal (i.n.) route. The figure below shows the RSV titer or viral load in the lung as determined by Taqman PCR. As can be seen from this figure, c-di-GMP administered intramuscularly attenuated the virulence of RSV and inhibited RSV infection (as measured by RSV titer/gene copies) in the lungs.



Number of RSV-L gene copies in lung cells recovered from mice immunized/infected as indicated. RNA was extracted from lung homogenates and cDNA was prepared to be used in reaction with RSV-L gene primers.

Activation of monocyte and granulocyte recruitment by Cyclic Dinucleotides Analogs:

Cyclic dinucleotides (including c-di-GMP, TBDMS-c-di-GMP, c-GpAp, cGpIp, cGpsGp) activated monocyte and granulocyte recruitment in mice. The *in vivo* recruitment of monocytes and granulocytes into the peritoneal cavity in response to cyclic dinucleotides is likely the outcome of local induction of certain chemokines (such as MCP-1) and the enhancement of adhesion molecules on either monocytes or endothelial cells. See Exhibit 1, page 5, column 1, lines 29-35 and column 2, lines 1-3 and data not shown. This data is consistent with the effects observed in the attenuation of

virulence, and the inhibition or reduction of microbial colonization and infection.

In conclusion, the wealth of experimental results presented above using very different established animal models of infection that mimic human or animal infection demonstrates very clearly that cyclic dinucleotides can attenuate and inhibit microbial virulence, colonization and infection (as determined by decreased colonization or by decreased disease and levels of infection). The results span microbial pathogens that include gram positive and gram negative bacterial pathogens, fungal pathogens and viral pathogens. Accordingly, a person of skill in the art would readily believe and expect that the presently claimed invention is applicable to the genus of microbial pathogens that includes bacteria, fungi and viruses and is therefore fully enabled.

The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such

willful false statements may jeopardize the validity of the application or any patent issued thereon.

/3 October 2008/	/David K.R. Karaolis/
Date	David K. R. KARAOLIS

October 2007

CURRICULUM VITAE

DAVID K. R. KARAOLIS, Ph.D.

Bacteriology Manager/Director
National Biodefense Analysis and Countermeasures Center (NBACC)
Frederick, MD 21702
Tel: 301-712 6057
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CITIZENSHIP United States

Security Clearance: SECRET- Current
DOJ Select Agent Clearance: Current
CDC Import and Transfer Permit: Etiologic Agents or Vectors of Human Disease- Current
USDA Import and Transport Permit: Controlled Materials and Organisms and Vectors- Current
Immunizations: Anthrax, Tularemia, BOT, Hep B, Tetanus

EDUCATION

1985	Matriculation	Newington College, Sydney, Australia
1986-1990	B. Sc.	Department of Life Sciences The University of Technology, Sydney, Australia
1990-1991	Honors	Department of Life Sciences The University of Technology, Sydney, Australia
1991-1994	Ph.D.	Department of Microbiology, The University of Sydney, Australia
1995-1998	Postdoctoral	Center for Vaccine Development, Department of Medicine University of Maryland School of Medicine/ VA Medical Center, Baltimore

SCIENTIFIC AND MANAGEMENT EXPERIENCE

Ph.D. Microbiologist (security clearance) with 20 years experience in a broad range of applied scientific fields including of clinical microbiology, microbial bioforensics, virulence assessment and characterization, molecular pathogenesis, antibiotics, vaccine and drug development. Experienced in laboratory procedures in the culture, identification, characterization and manipulation of both BSL-2 and BSL-3 select agents, including biothreat agents important in biodefense, as well as studying virulence assessment and host response using in vitro and in vivo animal models. Five (5) patents involving infectious disease, host response and therapeutics.

As the Director of Bacteriology at the National Biodefense Analysis and Countermeasures Center (NBACC), comprising both the National Bioforensic Analysis Center (NBFAC) and National Biothreat Characterization Center (NBTCC), I manage and direct bacteriology capabilities including bacterial diagnostics, applied research studies and the development of new technologies. My tenure has included the establishment of the NBACC/NBFAC bacteriology BSL-3 containment laboratory, as well as successfully obtaining ISO 17025 accreditation of bioforensic bacteriology operations under the ISO Quality Management System (QMS). Extensive

management experience in the strategic planning, coordination and program management of multidisciplinary national and international projects/programs and liason with government/private organizations, including the design and implementation of project goals, allocation of budget/resources, data analysis and reporting.

In addition, I have also pioneered the discovery and development of a novel drug-platform technology for commercialization. This work involves immunomodulator molecules and includes several patentable technologies and clinical applications including new immunoprophylactic, immunotherapeutic and vaccine approaches for preventing and treating infectious diseases and cancer.

AWARDS

1998-2003	Burroughs Wellcome Fund Career Award in the Biomedical Sciences
2005-2006	Burroughs Wellcome Fund Career Award (Supplemental award)
2006	Department of Homeland Security- Certificate of Recognition

EMPLOYMENT

2006-present	Bacteriology Manager/Director National Bioforensic Analysis Center (NBFAC) National Biodefense Analysis and Countermeasures Center (NBACC)
2006	Assistant Professor (adjunct) Department of Pediatrics University of Maryland School of Medicine
1999-2006	Assistant Professor Department of Epidemiology and Preventive Medicine University of Maryland School of Medicine
1999-present	Faculty Member (affiliate) Molecular and Cell Biology Graduate Program University of Maryland School of Medicine
1999-present	Assistant Professor (affiliate) Department of Medicine University of Maryland School of Medicine
1998-1999	Instructor of Medicine Division of Hospital Epidemiology University of Maryland School of Medicine
1995-1998	Postdoctoral Fellow Center for Vaccine Development University of Maryland School of Medicine
1991-1995	Clinical Microbiologist Department of Microbiology Hanly Moir Private Pathology Laboratories, Sydney
1991-1994	Research Assistant Department of Microbiology

The University of Sydney

1991-1994 Tutor/Teacher
Department of Life Sciences
The University of Sydney

1990-1991 Clinical Microbiologist
Department of Microbiology
The Royal North Shore Hospital, Sydney

1990-1991 Laboratory Demonstrator
Department of Microbiology
University of Technology, Sydney

1987-1989 Trainee Microbiologist
Department of Microbiology
Royal North Shore Hospital, Sydney

PROJECT MANAGEMENT COURSES

2002 Burroughs Wellcome Fund and Howard Hughes Medical Institute Course in Scientific Management

PROFESSIONAL ASSOCIATIONS

1988-present American Society for Microbiology
1988-present Australian Society for Microbiology
1998-present American Academy for the Advancement of Science

EDITORIAL TASKS

1996-present Ad Hoc Reviewer, Royal Society of Tropical Medicine and Hygiene
1999-present Ad Hoc Reviewer, Trends in Microbiology
1999-present Ad Hoc Reviewer, Infection and Immunity
2001-present Ad Hoc Reviewer, Journal of Antimicrobial Chemotherapy
2002-present Ad Hoc Reviewer, Microbiology
2002-present Ad Hoc Reviewer, Journal of Clinical Microbiology
2002-present Ad Hoc Reviewer, Journal of Infectious Diseases
2003-present Ad Hoc Reviewer, Molecular Microbiology

GRANT REVIEW WORK

1995-1998 USAID Office of Health and Nutrition
2003 The Wellcome Trust (United Kingdom)
2004 Science Foundation Ireland (SFI)
2005 U.S. Department of the Army

PATENTS

- Bacteriophage-based vaccines and detection systems, methods of using same, and products thereof.
Karaolis, D.K.R. U.S. Serial # 60/133373
- Method and system for direct detection of fungal pathogens.

Karaolis, D.K.R. U.S. Serial # 60/545,895
- Method for attenuating virulence of microbial pathogens and for inhibiting microbial biofilm formation.

Karaolis, D.K.R. PCT/US04/23498
- Method for stimulating the immune, inflammatory or neuroprotective response.

Karaolis, D.K.R. U.S. 11/079,886; PCT/US05/08447
- A method for inhibiting cancer cell proliferation or increasing cancer cell apoptosis.

Karaolis, D.K.R. U.S. 11/079,779; PCT/US05/08448

UNIVERSITY OF MARYLAND COMMITTEES AND ACTIVITIES

University of Maryland Committees:

2000–present	UMD Recombinant DNA Committee
2000–present	UMD Institutional Bio-Safety Committee

School of Medicine Committees:

1999-2000	Scientific Review Committee on NIH Program Project, Molecular and Cellular Pathogenesis of Urinary Tract Infection, J. Warren , Principle Investigator.
1999-2000	Scientific advisory committee for Health Sciences Facility II
1999	Judge for Graduate Research Conference Day
2000-2003	Alt. Representative for Faculty Council

Departmental Committees:

1999-2004	Research Committee, DEPM
1999-2002	Seminar Committee, DEPM
2001-2002	Graduate Admissions Committee, DEPM
2002-2003	Resource Allocation for Teaching and Service Committee, DEPM

VETERANS AFFAIRS COMMITTEES:

2002-present	Biosafety Committee, VA Medical Center, Baltimore

TEACHING ACTIVITIES

Teaching at University of Maryland School of Medicine:

1999-present	Bacterial Genetics MMIC/DMIC 635 (Graduate Students)

Teaching at other universities:

1990-1991	Clinical Microbiology Department of Microbiology University of Technology, Sydney
1991-1994	Microbiology

Department of Life Sciences
The University of Sydney

MENTORSHIP at UMB

Instructors (Faculty):

Afsar Ali, Ph. D. (2000-2003)

Postdoctoral fellows:

Jing Wang, M.D., Ph. D. (1999-9/2001)
Dalin Zhang, Ph.D. (1999-2003)
Afsar Ali, Ph. D. (2000)
Rajanna Chythanya (2001-2005)

Graduate students:

Mohammed Harun Rashid (2000-present)

Ph.D. Rotation

Amanda King (2000) – MCB Program
Jessina McGregor (2002) – DEPM Program
Simone Shurland (2003) DEPM program

Ph.D. Committee Member

Christopher J. Grim (Advisor: Rita R. Colwell)

UMD Research Training Program

Layla Lavasani (2002) – NIEHS (Minority) Toxicology Program, UMB
Tamara Webster (2003) - NIEHS (Minority) Toxicology Program, UMB
Keisha Findley (2003-2004) – MARC (Minority) Program, UMBC
Tara Brinck (2004) – Fogarty Minority International Training Program, UMBC

INVITED TALKS

1998 Karolinska Institute, Stockholm, Sweden. Analysis of the enteropathogenic *E. coli* LEE pathogenicity island: RDEC as a model.

1998 University of Sydney, Dept. of Microbiology. Genetic analysis of the *Vibrio* pathogenicity island.

1999 99th General Meeting of the American Society for Microbiology, Chicago, IL. Session: Phage and virulence; A bacteriophage encoding a pathogenicity island and type IV pilus in *V. cholerae*.

1999 16th Biennial conference on Virus and Phage Assembly. Rio Rico, AZ. A bacteriophage encoding a pathogenicity island and type IV pilus in *Vibrio cholerae*.

1999 XX SBM Congress, Brazilian Society for Microbiology, Salvador, Brazil. Genetics of virulence and evolution of *Vibrio cholerae*.

1999 XX SBM Congress, Brazilian Society for Microbiology, Salvador, Brazil. The *Vibrio cholerae* pathogenicity island.

1999 48th Annual Meeting of the American Society for Tropical Medicine and Hygiene, Washington, DC. Cholera and phage: genetic rearrangements, the *Vibrio* pathogenicity island, and prospects for emergence of new pandemic strains.

1999 FDA, Bethesda, Maryland. Epidemic cholera and phage: Role of phage in epidemic cholera.

2000 19th Annual Meeting of the American Society for Virology, Fort Collins, CO. Session: Viral virulence, pathogenesis and immunity; Bacteriophage-encoded virulence factors in *V. cholerae*.

2000 100th General Meeting of the American Society for Microbiology, Los Angeles, CA. Session: Interacting DNA elements, pathogenesis, and bacterial apoptosis; Virulence-conferring phage in *Vibrio cholerae*.

2002 Southwestern branch of the American Society for Microbiology, Annual Meeting, Gainsville, Fl. Session: Food Microbiology: Epidemic *V. cholerae*: PAIs, polysaccharide and persistence

2002 Thomas Jefferson University, Dept. of Biochemistry. Epidemic *V. cholerae*: Pathogenicity islands, polysaccharides and persistence.

2003 University of Sydney, School of Molecular Biosciences. Epidemic *V. cholerae*: Pathogenicity islands, polysaccharides and persistence.

2003 University of New South Wales, School of Biotechnology and Biomolecular Sciences. Epidemic Cholera: Importance of Pathogenicity islands and Exopolysaccharides.

2004 Johns Hopkins Hospital Bloomberg School of Public Health. *Vibrio cholerae* molecular pathogenesis.

2004 Catholic University of America, Department of Biology. *Vibrio cholerae* pathogenesis: new molecular insights and identification of a novel class of signaling (therapeutic?) molecule.

2004 Nabi Biopharmaceuticals. Cyclic Dinucleotides: a Novel Drug-Platform.

2005 Schering Plough. Cyclic Dinucleotides: a Novel Drug-Platform

2006 1st World Congress: Alliance for the Prudent use of Antibiotics (APUA). Antibiotic resistance in bioterror threats. Boston, MA. December 11-12.

ABSTRACTS

1. **Karaolis, D.K.R.**, R. Lan, PR. Reeves. 1994.Annual Meeting of the Australian Society for Microbiology, Melbourne, Victoria, Australia. Molecular evolution of the 7th pandemic clone of *Vibrio cholerae* and its relationship to other pandemic and epidemic strains. (Oral).

2. **Karaolis, D.K.R.**, R. Lan, PR. Reeves. 1995. 95th General Meeting of the American Society for Microbiology. Washington, DC. The 6th and 7th cholera pandemics are independent clones derived from environmental, nontoxigenic, non-O1 *Vibrio cholerae*.

3. **Karaolis, D.K.R.**, T.K. McDaniel, and E.C. Boedeker. 1995. Cloning of the RDEC-1 locus of enterocyte effacement (LEE) and functional analysis of its phenotype on Hep-2 cells. *Advances in Experimental Medicine and Biology*. Proceedings of the First International Rushmore Conference on Mechanisms in the Pathogenesis of Enteric Diseases, Mt. Rushmore, SD. Plenum Press. p241-245.

4. **Karaolis, D.K.R.**, T.K. McDaniel, J.B. Kaper, and E.C. Boedeker. 1996. Cloning of the RDEC-1 locus of enterocyte effacement (LEE) and functional analysis of the phenotype on HEp-2 cells. 96th General Meeting of the American Society for Microbiology. New Orleans, LA. Abstract B-90.

5. **Karaolis, D.K.R.**, R.Lan, P.R. Reeves. 1997. The *aldA* gene of *Vibrio cholerae* is a genetic marker for strains with pandemic potential. From the Proceedings of the 31st U.S.-Japan Joint Conference on Cholera and Related Diarrheal Disease, Kiawah Island, South Carolina, USA. 1995. *In*: Cytokines, Cholera, and the gut. G.T. Keusch and M. Kawakami *Eds*. IOS Press.p213-217.

6. **Karaolis, D.K.R.**, S. Sozhamannan, J.A. Johnson, J.B. Kaper. 1998. 98th General Meeting of the American Society for Microbiology. Atlanta, GA. Novel non-O1/non-O139 *Vibrio cholerae* containing the VPI and CTX. Abstract B-179.

7. Lipp, E.K., I.N.G. Rivera, M. Talledo, A. Neale, **D.K.R. Karaolis**, A. Huq, R.R. Colwell. 2001. 101st General Meeting of the American Society for Microbiology. Orlando, FL. Optimal conditions for infection and multiplication of *Vibrio cholerae* specific phages isolated from seawater.

8. Vital-Brazil, J.M., **D.K.R. Karaolis**, D.P. Rodrigues, L.C. Campos. 2001. 101st General Meeting of the American Society for Microbiology. Orlando, FL. Prevalence of virulence-associated genes in clinical and environmental *Vibrio cholerae* strains isolated in Brazil between 1991-1999.

9. Wang, J. J. Xu, A. Ali, **D.K.R.Karaolis**. 2001. 101st General Meeting of the American Society for Microbiology. Orlando, FL. Genetic analysis of the plasmid form of the *Vibrio cholerae* pathogeneicity island.

10. Zhang, D. S. Rao, **D.K.R. Karaolis**. 2001. 101st General Meeting of the American Society for Microbiology. Orlando, FL. Functional analysis of Orf4 encoded by the *Vibrio cholerae* pathogenicity island.

11. Zhang, D., W. Sun, Z. Xu, **D.K.R. Karaolis**. 2002. 102st General Meeting of the American Society for Microbiology. Salt Lake City, UT. The VPI-encoded Orf4 modulates secreted proteins in *Vibrio cholerae*.

12. Rashid, M. H., A. Ali, **D.K.R. Karaolis**. 2002. 102st General Meeting of the American Society for Microbiology. Salt Lake City, UT. Analysis of the Genetic Switch for Phenotypic Conversion Between the Smooth and Rugose Exopolysaccharide Phenotypes of *V. cholerae.*

13. Rashid, M. H., A. Ali, **D.K.R. Karaolis**. 2003. 103st General Meeting of the American Society for Microbiology. Washington, D.C. Genetic analysis of high frequency rugose exopolysaccharide production (HFRP) in epidemic *V. cholerae.*

14. Rajanna, C. and **D.K.R. Karaolis**. 2003. 103st General Meeting of the American Society for Microbiology. Washington, D.C. The VPI-encoded Int and VpiT of epidemic *V. cholerae* have roles in high frequency rugose exopolysaccharide production (HFRP).

15. Zhang, D., Sun, W. and **D. K. R. Karaolis**. 2003. 103st General Meeting of the American Society for Microbiology. Washington, D.C. The *Vibrio* pathogenicity island *mop* modulates cholera toxin, motility and biofilm formation in epidemic *V. cholerae.*

16. Rajanna, C., Rashid, M.H. and **D.K.R. Karaolis**. 2004. 104th General Meeting of the American Society for Microbiology. New Orleans. Regulation of *Vibrio cholerae* biofilm formation and intestinal colonization by *Vibrio* pathogenicity island recombinases.

17. Zhang, D., Rajanna, C. and **D.K.R. Karaolis**. 2004. 104th General Meeting of the American Society for Microbiology. New Orleans. Recombinase-mediated control of cholera toxin in epidemic *Vibrio cholerae.*

18. **Karaolis, D.K.R.**, Rashid, M.H. Rajanna, C., Buckles, E., Luo, W. and Hayakawa, Y. 2004. 44th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC). Washington, D.C. c-di-GMP as a novel anti-biofilm agent against *Staphylococcus aureus.*

19. **Karaolis, D.K.R.**, Means T.K., Brouillette, E., Talbot, B.G., Yang, D., Muraille, E., Hyodo, M., Hayakawa, Y. and Malouin, F. 2006. General meeting of the Amercan Society for Microbiology. Orlando. c-di-GMP is an immunostimulatory molecule with prophylactic and adjuvant activity.

PUBLICATIONS

BOOK CHAPTERS

1. **Karaolis, D.K.R.** and E.C. Boedeker. 1996. Enteric pathogens: Population genetics and pathogenesis of *Escherichia coli* and *Vibrio cholerae* infections. *In*: Gastrointestinal Microbiology. Vol. 2. R.I. Mackie and B.A. White *eds*. Chapman and Hall. Chapter 16, p622-657.

2. Bloom, P.D., **D.K.R. Karaolis**, E.C. Boedeker. 1997. *Escherichia coli* associated diarrhea. *In*: Gastrointestinal Infections. J.Thomas-LaMont *ed.* Marcel Dekker. Chapter 15, p 453-498.

3. **Karaolis, D.K.R.** and J.B. Kaper. 1999. Pathogenicity islands and other mobile virulence elements of *Vibrio cholerae*. *In*: Pathogenicity islands and Other Mobile Virulence Elements. J.B. Kaper and J. Hacker *eds*. ASM Press. Chapter 9, p167-187.

4. **Karaolis, D.K.R.** 2001. Pathogenicity islands. *In*: The Encyclopedia of Genetics. S. Brenner and J.M. Miller *eds*. Academic Press.

JOURNALS (peer reviewed)

1. **Karaolis, D.K.R.**, Lan, R. and Reeves, P.R. 1994. Sequence variation in *Shigella sonnei* (Sonnei), a pathogenic clone of *Escherichia coli*, over four continents and 41 years. *J. Clin. Microbiol.* 32:796-802.

2. **Karaolis, D.K.R.**, Lan, R. and Reeves, P.R. 1994. Molecular evolution of the seventh-pandemic clone of *Vibrio cholerae* and its relationship to other pandemic and epidemic *V. cholerae* isolates. *J. Bacteriol.* 176: 6199-6206.

3. **Karaolis, D.K.R.**, Lan, R. and Reeves, P.R. 1995. The sixth and seventh cholera pandemics are due to independent clones separately derived from environmental, nontoxigenic, non-O1 *Vibrio cholerae*. *J. Bacteriol.* 177:3191-3198.

4. **Karaolis, D.K.R.**, Johnson, J.A., Bailey, C.C., Boedeker, E.C., Kaper, J.B., and Reeves, P.R. A *Vibrio cholerae* pathogenicity island associated with epidemic and pandemic strains. 1998. *PNAS.* 95:3134-3139.

5. Pupo, J., **Karaolis, D.K.R.,** Lan, R. and Reeves, P.R. 1997. Evolutionary relationships among pathogenic and non-pathogenic *Escherichia coli* inferred by MLEE and *mdh* sequence studies. *Infect. Immun.* 65:2685-2692.

6. **Karaolis, D.K.R.**, Somara, S., Maneval, D.R., Johnson, J.A., Kaper, J.B. 1999. A bacteriophage encoding a pathogenicity island, a type-IV pilus and a phage receptor in cholera bacteria. *Nature.* 399:375-379.

7. **Karaolis, D.K.R.**, Lan, R., Kaper, J.B., Reeves, P.R. 2000. A comparison of the *Vibrio cholerae* pathogenicity islands in 6th and 7th pandemic strains. *Infect. Immun.* 69: 1947-1952.

8. Vital Brazil, J.M., Alves, R.M., Rivera, I.N.G., Rodrigues, D.P., **Karaolis, D.K.R.** and Campos, L.C. 2002. Prevalence of virulence-associated genes in clinical and environmental *Vibrio cholerae* strains isolated in Brazil between 1991-1999. *FEMS Microbiol. Lett.* 215:15-21.

9. Ali, A., Rashid, M. H. and **Karaolis, D.K.R.**. 2002. High frequency rugose exopolysaccharide production in *Vibrio cholerae*. *Appl. Environ. Microbiol.* 68:5773-5778.

10. Zhang, D. Sun, W., Xu, Z. and **Karaolis, D.K.R.** 2003. The VPI-encoded Mop modulates the pathogenesis and reactogenicity of epidemic *Vibrio cholerae in vivo*. *Infect Immun.* 71:510-515.

11. Talledo, M., Rivera, I.N.G., Lipp, E. K., Neale, A., **Karaolis, D.K.R.**, Huq, A., and Colwell, R. R. 2003. Characterization of a *Vibrio cholerae* phage isolated from the coast of Peru. *Environ. Microbiol.* 5:350-354.

12. Zhang, D., Sun, W. and Karaolis, D.K.R.. 2003. Analysis of the *Vibrio* pathogenicity island-encoded Mop protein suggests a pleiotropic role in the virulence of epidemic *Vibrio cholerae*. *FEMS Microbiol. Lett.* 225:311-318.

13. Rashid, M. H., Rajanna, C., Ali, A. and **Karaolis, D. K. R.** 2003. Identification of genes involved in the switch between the smooth and rugose phenotypes of *Vibrio cholerae*. FEMS Microbiol. Letts. 227:113-119.

14. Chythanya, R. Wang, J. Zhang, D. Xu, Z., Ali, A., Hou, Y-M. and **Karaolis, D.K.R.** 2003. The *Vibrio* pathogenicity island of epidemic *Vibrio cholerae* forms precise extrachromosomal circular excision products. *J. Bacteriol.* 185: 6893-6901.

15. Rashid, M.H., Rajanna, C., Zhang, D., Magder, L.S., Ali, A., Dumontet, S., **Karaolis, D. K. R.** 2003. Role of exopolysaccharide, the rugose phenotype and VpsR in the pathogenesis of epidemic *Vibrio cholerae.* FEMS Microbiol. Lett. 230:105-113.

16. Zhang, D.L., Manos, J., Belas, R and **Karaolis, D.K.R.** 2004. Transcriptional analysis and operon structure of the *tagA-orf2-orf3-mop-tagD* region on the *Vibrio* pathogenicity island in epidemic *V. cholerae.* FEMS Microbiol. Lett. 235:199-207.

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